

Pigment Fluorescence Signatures as an Index to the Taxonomic Structure of Phytoplankton Communities

Dr. Gary Hitchcock
Marine Biology and Fisheries
Rosenstiel School of Marine and Atmospheric Science
4600 Rickenbacker Cswy.
Miami, FL 3149
phone: (305) 361-4926 fax: (305) 361-4765 email: ghitchcock@rsmas.miami.edu

Dr. Kenneth Voss
Physics Department
College of Arts and Sciences
University of Miami
Coral Gables, FL 33146
phone: (305)284-2323 fax: (305) 284-4222 email: kvoss@miami.edu

Award Number: N000140110284
<http://www.rsmas.miami.edu/divs/mbf/people/ghitchcock.html>

LONG-TERM GOALS

The taxonomic composition of phytoplankton communities is a major determinant of the size structure of trophic levels in marine ecosystems. Oligotrophic waters are typically dominated by small picoplankton (cyanobacteria) that support the microbial loop. Phytoplankton in more productive coastal waters, in contrast, are frequently dominated by larger chromophytes such as diatoms, dinoflagellates and prymnesiophytes that support larger herbivores. A major goal of biological oceanographers is to map the spatial distributions of taxonomic groups in natural plankton communities and relate this knowledge to the structure and functioning of marine ecosystems.

Microscopic methods cannot detect all picoplankton in natural populations. Flow cytometry and molecular techniques derived from biomedical research are now being applied to natural populations to describe taxonomic characteristics of phytoplankton communities (Long, 1998). These techniques, however, are not yet capable of continuously mapping spatial distributions in situ. The distinct pigment composition of individual phytoplankton taxonomic classes can be used as pigment 'biomarkers' to qualitatively map the abundance of phytoplankton classes with high performance liquid chromatography, but these methods are also not amenable to continuous mapping of natural populations.

Distinct fluorescence spectra of phytoplankton classes can be utilized to identify and map the distribution of dominant phytoplankton groups in natural communities at a variety of spatial scales (Yentsch and Yentsch, 1979). The long term objective of this project is to develop a multi-wavelength in situ, autonomous fluorometer capable of mapping spatial distributions of phytoplankton taxonomic groups (mainly chromophytes and cyanobacteria) at length scales of 10s meters to kilometers in the horizontal, and ultimately meters in the vertical. These length scales correspond to the dominant scales

of spatial variability of phytoplankton in coastal waters. The test region for the prototype instrument is Florida Bay, a shallow subtropical lagoon, and the contiguous shelf waters. Cyanobacterial blooms frequently occur in the north central Bay (Fig. 1, left panel) with diatoms dominant in the western Bay (Fig. 1, right panel).

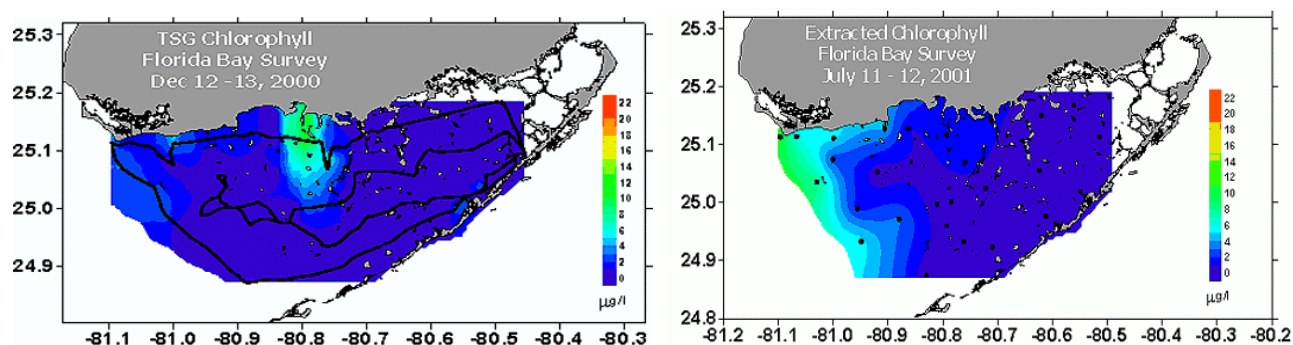


Figure 1. The spatial distribution of chlorophyll *a* in Florida Bay during a cyanobacteria bloom (December, 2000, left panel) and a diatom bloom (July 2001, right panel). Peak concentrations in the north central Bay during *Synechococcus* blooms can exceed 20 $\mu\text{g l}^{-1}$. The pigment maximum in the western Bay in July 2001 corresponds to a bloom dominated by *Rhizosolenia* and other large centric diatoms, with maximum chlorophyll *a* concentrations of 10 $\mu\text{g l}^{-1}$.

OBJECTIVES

1. Configure an optical system and construct an autonomous, low-power multiple-channel fluorometer.
2. Evaluate the capability of matrices of fluorescence signatures to serve as indices to chromophytes and cyanobacteria in Florida coastal waters. The validity of fluorescence signatures will be assessed from RP-HPLC analyses provided by a colleague with a concurrent research effort in the Bay.
3. Map the temporal and spatial pattern of fluorescence signatures in a subtropical lagoon, Florida Bay, and contiguous shelf waters.

APPROACH

Fluorescence excitation and emission characteristics (fluorescence ‘signatures’) have been utilized as as indices to the taxonomic structure of freshwater and coastal phytoplankton populations for more than a decade. Although it was initially proposed that fluorescence spectral responses could potentially discriminate among taxonomic groups such as diatoms, dinoflagellates and coccolithophrids (Yentsch and Yentsch, 1979), fluorescence signatures of four taxonomic groups can be readily distinguished. These signatures correspond to chromophytes (‘golden-brown’ algae), chlorophytes (‘green’ algae), cryptophytes and rhodophyceae, and cyanobacteria (‘blue-green’ algae). In golden brown algae, the carotenoids transfer energy to chlorophyll *a*, and although they do not fluoresce, the resulting chlorophyll *a* fluorescence can be used as an index to the presence of chromophyte accessory pigments.

Indices such as the chlorophyll accessory pigment (CAP) ratio have been utilized as a means to distinguish chromophytes, chlorophytes and cyanobacteria in marine waters based on the fluorescent response $F_{530:685}/F_{450:685}$, where $F_{\text{ex:em}}$ corresponds to the fluorescence response at paired excitation (ex) and emission (em) wavelengths, respectively. Subsequently, Watras and Baker (1988) developed a ratio ($F_{630:660}/F_{430:680}$) that successfully distinguishes freshwater cyanobacteria from other taxonomic groups, while Seppälä and Balode (1998) and Cowles et al (1993) have also mapped cyanobacterial distributions in coastal waters based on multiple wavelengths. We are using a four component matrix with two excitation and two emission wavelengths to identify cyanobacteria and chromophyte populations.

One factor complicates the use of fluorescence spectra as a means to distinguish the taxonomic composition of natural phytoplankton populations. Fluorescence signatures of phytoplankton are influenced by physiological responses to changing environmental conditions. Factors such as light, nutrient status and cellular optical properties influence fluorescence excitation-emission spectra (e.g., Soo Hoo *et al.*, 1986; Lutz et al., 2001). Thus fluorescence signatures in our study are being evaluated with respect to concurrent measurements of accessory pigments to validate spatial distributions and the taxonomic composition of natural populations.

WORK COMPLETED

A dual-wavelength flow-through fluorometer has been completed and its response evaluated with laboratory cultures of diatoms, dinoflagellates, and cyanobacteria (*Synechococcus* spp. and *Prochlorococcus marinus*) cultures. The prototype electronics were constructed by BathySystems, Inc. and the University of Miami, with the optics based on an autonomous fluorometer developed for Lagrangian platforms (Hitchcock et al., 2000). The system contains a controller board with a Motorola microcontroller that regulates the excitation light sources and data collection from solid state photodiodes.

Initially we attempted to integrate the fluorometer with an existing data collection system onboard a boat that conducts monthly surveys of Florida Bay. That was unsuccessful, due to the limitation of the data collection system. Dr. K. Voss has subsequently integrated the fluorometer with a GPS receiver through a laptop computer. The data logging system (Windmill) on the laptop computer provides for ports for recording ancillary data, such as conductivity, temperature, transmittance or other properties.

RESULTS

We have validated the fluorometer response in terms of spectral responses of the taxonomic groups of interest (chromophytes and cyanophyceae) for the two major phytoplankton groups that dominate natural communities in Florida Bay. The fluorescence spectra were measured with laboratory cultures and compared to those from a Hitachi F4500 fluorescence spectrofluorometer. Our results confirm Poryvkina *et al.* (1994), who described spectral signatures of 28 phytoplankton species in terms of 2-dimensional excitation-emission matrices.

The field phase of this effort is now beginning. Maps of fluorescence distributions are derived from monthly surveys of Florida Bay conducted by NOAA's South Florida Ecosystem Restoration Prediction and Modeling (SFERPM) program. Ancillary physical (temperature, salinity, position) and

bio-optical (chlorophyll *a* and CDOM fluorescence, beam transmittance) data are collected during the survey with position, and will be analyzed to determine spatial relationships of plankton distributions to physical and biological parameters. Individual samples are also collected from the various basins for accessory pigments through R-HPLC analyses by Dr. W. Louda, FAU (Louda *et al.*, 1998).

IMPACT/APPLICATIONS

The principal test region for the dual wavelength fluorometer is a subtropical lagoon that is the focus of a large interagency-funded research effort to evaluate scenarios for various restoration activities in the Everglades (details on Everglades Restoration are at: <http://www.aoml.noaa.gov/flbay/index.html>). The multi-channel fluorometer will provide spatial maps of taxonomic groups within the Bay and adjacent shelf. Knowledge of the diatom (chromophyte) and cyanobacterial distributions, and their scales of variability, will provide information on how physical and biological factors influence phytoplankton dynamics in the Bay.

TRANSITIONS

There are no current transitions.

RELATED PROJECTS

There is one related study of the structure of plankton communities in Florida Bay. Dr. W. Louda of Florida Atlantic University is describing the structure of phytoplankton and benthic microalgal communities through high performance liquid chromatographic assays, emphasizing the cycling of plant pigments in the water column and benthos. The central goal of his study is to determine the role of pelagic and benthic microalgae in the structure and functioning of food webs in Florida Bay.

REFERENCES

Bidigare, R.R., B.B. Prézelin, and R.C. Smith. (1992). Bio-optical models and the problem of scaling. In: P. G. Falkowski and A. D. Woodhead (eds.). *Primary Productivity and Biogeochemical Cycles in the Sea*. Plenum Press. New York. pp. 175-212.

Cowles, T.J., R.A. Desiderio, and S. Neuer. 1993. In situ characterization of phytoplankton from vertical profiles of fluorescence emission spectra. *Mar. Bio.* 115: 217-222.

Hitchcock, G. L., E.L. Key, and J. Masters. 2000. The fate of upwelled waters in the Great Whirl, August, 1995. *Deep-Sea Res. II.* 47:1605-1621.

Kirk, J. T. O. 1994. *Light and photosynthesis in aquatic ecosystems*. 2nd Ed. Cambridge University Press. Cambridge.

Long, E. F. 1998. Molecular Phylogenetics: New perspectives on the ecology, evolution, and biodiversity of marine organisms. In: K. E. Cooksey (ed.). *Molecular Approaches to the Study of the Ocean*. Chapman and Hall. London. pp. 1-27.

Louda, J. W., J. Li, L. Liu, M. N. Winfree, and E. A. Baker. 1998. Chlorophyll-a degradation during senescence and death. *Org. Geochemistry*. 29:1233-1251.

Lutz, V.A., S. Sathyendranath, E.J.H. Head, and W.K.W. Li. 2001. Changes in the in vivo absorption and fluorescence excitation spectrum with growth irradiance in three species of phytoplankton. *J. Plankton Res.* 23: 555-569.

Poryvkina, L., S. Babichenko, S. Kaitala, H. Kuosa, and A. Shalapjonk. 1994. Spectral fluorescence signatures in the characterization of phytoplankton community composition. *J. Plankton Res.* 16: 1315-1327.

Seppälä, J., and M. Balode. 1998. The use of spectral fluorescence methods to detect changes in the phytoplankton community. *Hydrobiologia*. 363: 207-219.

Soo Hoo, J. B., D. A. Kiefer, D. J. Collins, and I. S. McDermid. 1986. In vivo excitation and absorption spectra of marine phytoplankton. I. Taxonomic characteristics and responses to photoadaptation. *J. Plankton Res.* 8: 197-214.

Watras, C. J., and A. L. Baker. 1988. Detection of planktonic cyanobacteria by tandem in vivo fluorometry. *Hydrobiologia*. 169: 77-84.

Yentsch, C. S., and C. M. Yentsch. 1979. Fluorescence spectral signatures: The characterization of phytoplankton populations by the use of excitation and emission spectra. *J. Mar. Res.* 37: 471-483.

PUBLICATIONS: No publications have yet been submitted.

PATENTS: There are no patents from this work.